

Proanthocyanidin from Persimmon Fruits

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Reprinted from

Journal of the Chinese Chemical Society

Ser. II, Vol. 14, No. 1

March, 1967

PUBLISHED BY THE SOCIETY, TAIPEI, TAIWAN
THE REPUBLIC OF CHINA

Short Communication

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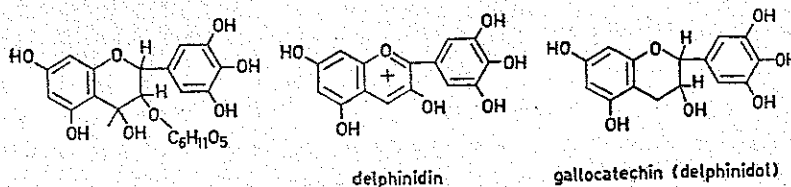
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(Received March 17, 1967)

Degradative and other data described below have shown that the tannin of unripe fruits of persimmon (*Diospyros virginiana*) actually contains (+)-gallo catechin unit in the structure and must be regarded as condensed flavonoid pigments, probably of similar structure to the polymeric proanthocyanidin from avocado seed.

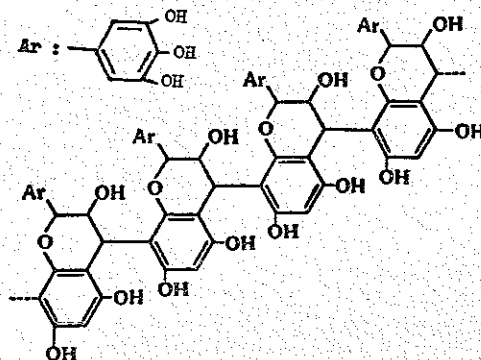
The possible structure of the proanthocyanidin from persimmon fruits is proposed tentatively as a polymer of 5, 7, 3', 4', 5'-pentahydroxyflavanonol.

Investigations by Mandel *et al.*,¹ demonstrating that the unripe fruits of persimmon contain a powerful inhibitor of cellulase, prompted us to investigate the nature of the active principle. Several kinds of proanthocyanidin have been recently reported in cranberries,² cola nut,² hawthorn berries,³ avocado seed⁴ and strawberry leaves.⁵ The proanthocyanidin of Japanese persimmon (*Diospyros kaki* L. Ebenaceae) was studied by Ito and Oshima,⁶ and the main constituent was proposed to be a polymer of leucodelphinidinglycoside.



The structure of American persimmon (*Diospyros virginiana*) tannin is not yet known although a short communication by Ito and Joslyn has appeared.⁷

By mild hydrolysis of the proanthocyanidin from fruits of American persimmon delphinidin, epigallocatechin and galocatechin (delphinidol) are isolated. The possible structure of the principle of the persimmon inhibitor is proposed tentatively as the following structure.



Experimental Section

Isolation of the proanthocyanidin: Unripe persimmon (*Diospyros virginiana*) fruits (collected in early August) were blended in a Waring blender with 2-1/2 times their weight of methanol. The suspension was filtered through cloth, and the filtrate centrifuged at 10,000 rpm. To the clear solution (100 ml) was added 25 ml M/1 K_2HPO_4 . The precipitate was washed with methanol, and dried from acetone. Yield 6g per 100 grams fresh weight of persimmon.

Purity of the proanthocyanidin: The light pink amorphous powdered proanthocyanidin was chromatographed on silica gel (TLC) with ethanol, ethanol-chloroform (2:1), ethanol-ether-*n*-hexane (3:1:1) and acetone-ether (3:1) as solvent systems; three components with a slight degree of tailing were identified as galocatechin, epigallocatechin and delphinidin.

Extraction with ethyl acetate: The proanthocyanidin (1.5g) was extracted continuously with ethyl acetate for two weeks. The solvent was removed *en vacuo*. The solid residue (45mg) was identified by paper chromatography as gallo catechin and delphinidin. Yield 0.3%.

Hydrolysis of proanthocyanidin with mineral acids: The proanthocyanidin was hydrolyzed by heating 200mg of the material with strong acid (HCl or H₂SO₄; 100ml of 70% HCl or 0.05 N-HCl) for 1 hour on a boiling-water bath. After cooling the solution was extracted with isoamylalcohol (50ml x 3). The red pigment was identified by chromatography and infrared spectrum as delphinidin.

The aqueous phase, after extraction of the pigment, was neutralized and treated with lead acetate and sodium phosphate. Chromatographic analysis of the resultant solution failed to reveal the presence of sugars or catechin.

Treatment with sulfurous acid: Proanthocyanidin (1g) was dissolved in water (80ml) and sulfur dioxide was bubbled for several minutes. This was followed by heating on a boiling-water bath with continued bubbling of sulfur dioxide for 2 hours. After cooling, the solution was extracted with ethyl acetate and amylalcohol yielding gallo catechin, (26%) m.p. 185-190° and delphinidin (16%), respectively. (Lit.⁹ gallo catechin, m.p. 185-188°; (+)-casuarin¹⁰ 185-190°; (-)-epigallo catechin, 218°; (+)-catchin, 96°, 177°, 219°; (-)-epicatechin, 242°). Develop on paper using 2% CH₃COOH-H₂O, spray with 2% CH₃OH-FeCl₃, 2 spots: Rf 0.3 and 0.4 violet. (Lit.¹¹ (-)-epigallo catechin Rf 0.31, (+)-gallo catechin Rf 0.40). Delphinidin, AcOH-HCl-H₂O (5:1:5) Rf 0.25 (Lit.¹² 0.22).

Acknowledgment

The authors thank Dr. George P. Dateo for invaluable discussion and technical help and Prof. K. Hayashi and Prof. Y. Takino for their kind supply of authentic anthocyanidin and

epigallocatechin. One of us (F. C. C.), on leave of absence from National Taiwan University, gratefully acknowledge the award of Visiting Scientist Research Associateship from National Academy of Sciences-National Research Council which permitted initiation of this work. Financial support from the National Council on Science Development (the Chemistry Research Center) was also greatly appreciated.

Literature Cited

* Presented at the IUPAC 4th International Symposium on the Chemistry of Natural Products, Stockholm, June, 1966.

- (1) M. Mandels and E. T. Reese, *Ann. Rev. Phytopathology*, **3**, 85 (1965).
- (2) K. Weinges and K. Freudenberg, *Chem. Comm.*, 1965, 220.
- (3) K. Freudenberg and K. Weinges, *Angew. Chem.*, **74**, 182 (1962).
- (4) T. A. Geissman and H. F. K. Dittmar, *Phytochem.*, **4**, 359 (1956).
- (5) L. L. Creosy and T. Swain, *Nature*, **208**, 151 (1965).
- (6) S. Ito and Y. Oshima, *Agr. Biol. Chem.*, **26**, 156 (1962).
- (7) S. Ito and M. A. Joslyn, *Nature*, **204**, 475 (1964); *J. Agr. Food Chem.*, **12**, 511 (1964).
- (8) V. C. Quesnel, *Tetrahedron Letters*, 1964, 3699.
- (9) W. Mayer and G. Bauni, *Ann.*, **611**, 264 (1956).
- (10) Y. Oshima, *Agri. Chem. Soc. Japan*, **15**, 636 (1939).
- (11) D. E. Hathway, *Biochem. J.*, **70**, 34 (1958).
- (12) K. Hayashi and Y. Abe, *Botan. Mag. (Tokyo)*, **69**, 577 (1956); K. Hayashi, *Pharmazie*, **12**, 245 (1957); K. Hayashi, *The Chemistry of Flavonoid Compounds* (Ed. by T. A. Geissman), p. 275 (1962).